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Influence of *Yucca shidigera* Extract on Ruminal Ammonia Concentrations and Ruminal Microorganisms

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An extract of the desert plant *Yucca shidigera* was assessed for its possible benefit in ruminal fermentation. The extract bound ammonia in aqueous solution when concentrations of ammonia were low (up to 0.4 mM) and when the extract was added at a high concentration to the sample (20%, vol/vol). The apparent ammonia-binding capability was retained after autoclaving and was decreased slightly following dialysis. Acid-precipitated extract was inactive. No evidence of substantial ammonia binding was found at higher ammonia concentrations (up to 30 mM). When *Y. shidigera* extract (1%, vol/vol) was added to strained rumen fluid in vitro, a small (6%) but significant ($P < 0.05$) decrease in ammonia concentration occurred, apparently because of decreased proteolysis. Inclusion of *Y. shidigera* extract (1%, vol/vol) in the growth medium of the rumen bacterium *Streptococcus bovis* ES1 extended its lag phase, while growth of *Butyrivibrio fibrisolvens* SH13 was abolished. The growth of *Prevotella* (*Bacteroides*) *ruminicola* B_14 was stimulated, and that of *Selenomonas ruminantium* Z108 was unaffected. Protozoal activity, as measured by the breakdown of ^{14}C-leucine-labelled *S. ruminantium* in rumen fluid incubated in vitro, was abolished by the addition of 1% extract. The antimicrobial activities were unaffected by precipitating tannins with polyvinylpyrrolidone, but a butanol extract, containing the saponin fraction, retained its antibacterial and antiprotozoal effects. Saponins from other sources were less effective against protozoa than *Y. shidigera* saponins. *Y. shidigera* extract, therefore, appears unlikely to influence ammonia concentration in the rumen directly, but its saponins have antimicrobial properties, particularly in suppressing ciliate protozoa, which may prove beneficial to ruminal fermentation and may lead indirectly to lower ruminal ammonia concentrations.

Extracts of the *Yucca shidigera* plant have been used for many years in the food, feed, and cosmetics industries. The plant contains sarsaponin, a group of steroidal glycosides, which can influence ruminant productivity. Sarsaponin had beneficial effects on rumen fermentation with some diets but not others (13). Digestion of organic matter and starch tended to be stimulated (13, 28). Reports of the effects on nitrogen metabolism have been mixed: one study noted an increase in feed nitrogen degradation (13), others noted a decrease (8, 28), and others noted little or no effect (10, 29).

The apparent ability of *Y. shidigera* extract to bind ammonia has been utilized recently in the livestock industry to decrease odors from pig and poultry wastes, resulting in improved animal welfare and productivity (4, 15, 16). A limited amount of work has been done with ruminants, which suggests that *Y. shidigera* extract may also be useful in controlling odors from cattle manure (11). The ammonia-binding ability of *Y. shidigera* extract was not associated with the saponin fraction in a butanol extraction, indicating that other materials, which were named glycofractions, were involved in ammonia binding (16). Whole *Y. shidigera* extract decreased rumen ammonia concentrations in cows receiving a diet containing 1% urea (24) and in in vitro incubations with rumen fluid (10), and sarsaponin decreased the rumen ammonia concentration in some studies (8, 12).

Since ammonia overproduction in the rumen leads to inefficient N retention and, in severe cases, ammonia toxicity in the host animal (20), it appeared possible that *Y. shidigera* extract could have a role in regulating the release of ammonia in the digestive tract of ruminants. The present work was therefore undertaken to evaluate the ammonia-binding characteristics of *Y. shidigera* extract and its effects on ammonia concentrations and microbial activities in rumen fluid and in pure cultures of ruminal bacteria.

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MATERIALS AND METHODS

***Y. shidigera* extract.** The extract used in this study was the liquid form of Deodorase, which was supplied by Alltech, Inc., Nicholasville, Ky. Only one sample was used throughout the experiments.

Influence of *Y. shidigera* extract on ammonia estimation. Ammonia was assayed by the phenol-hypochlorite-nitroprusside method (32) as follows. For spectrophotometric determinations, 1.0 ml of phenol-nitroprusside reagent (containing 10 g of phenol and 0.05 g of sodium nitroprusside per liter) was added to 0.2 ml of sample in a glass test tube. The tube was vortexed, and 0.8 ml of alkaline hypochlorite solution (6.25 g of NaOH and 10.5 ml of sodium hypochlorite [10 to 14% available chlorine per liter; BDH] per liter) was added. The mixture was vortexed once more and incubated at 39°C for 15 min, and the A_{650} was read. Alternatively, the measurements were done in a plate reader with 1/10 of the volumes used for spectrophotometric determinations. The results of the spectrophotometric and plate reader methods were similar. To determine the effect of *Y. shidigera* extract, dilutions of the extract were added to the ammonia solution, and the phenol-hypochlorite solution was added approximately 10 min later. The effects of autoclaving the extract at 121°C for 15 min and dialyzing the extract against four changes of deionized water for 24 h at 4°C were determined by the same method.

Influence of *Y. shidigera* extract on ammonia concentration in aqueous solution. Progressively increasing concentrations of

Y. shidigera extract were added to 75 ml of buffer incubated in conical flasks at 39°C in a shaking water bath. The buffers used were 50 mM potassium phosphate (pH 7.5) or Clark and Lubs HCl-KCl (pH 2.0) solution (6). Five dialysis bags, made from Visking tubing (molecular size cutoff, 12 to 14 kDa; Medicell International Ltd., London, England) and containing 2 ml of buffer but no extract, were added to each flask. Ammonium chloride additions were made at hourly intervals, and the dialysis bags were removed, washed rapidly, and frozen at −20°C immediately before the addition of more ammonium chloride.

Influence of *Y. shidigera* extract on ammonia concentrations in rumen fluid. Every day, four mature cannulated sheep were fed 1 kg of a mixed diet of hay, barley, molasses, fishmeal, and vitamins-minerals (500, 299.5, 100, 91, and 9.5 g of dry matter, respectively, per kg) in two equal meals at 0900 and 1600 h. Rumen fluid was removed via the rumen cannula 3 h after feeding and strained through four layers of muslin. A portion was centrifuged at 120 × *g* for 10 min, and then the supernatant was centrifuged again at 41,000 × *g* for 20 min. This clarified rumen fluid was added to dialysis bags, 2.0 ml per bag, and five dialysis bags were added to conical flasks containing 75 ml of strained rumen fluid with 1% (vol/vol) *Y. shidigera* extract. The flasks were stoppered and then incubated in a shaking water bath at 39°C, and the bags were removed for ammonia analysis as described before. Samples of rumen fluid from the four sheep were incubated separately. In experiments with urea and casein, 30 and 100 mg, respectively, were added to conical flasks containing 50 ml of strained rumen fluid. Otherwise, the experiment was similar. The rumen fluid was kept under CO_2 throughout, and a flow of CO_2 was supplied to the conical flasks during sampling.

Influence of *Y. shidigera* extract on protozoal activity in rumen fluid. Samples of strained rumen fluid were prepared from four sheep receiving the same diet as that described before. The rumen fluid was kept warm in vacuum flasks and under CO_2 throughout. *Selenomonas ruminantium* Z108 was labelled with [^{14}C]leucine as described previously (31). The bacteria were washed in anaerobic 50 mM potassium phosphate buffer (pH 7.5) containing 2 mM cysteine and added to rumen fluid to which was also added 0.6 mg of unlabelled leucine per ml to prevent the reincorporation of label arising from the breakdown of *S. ruminantium* protein (31). *Y. shidigera* extract was added to the mixture at 0.1 or 1%, and the flasks were incubated with gentle shaking to maintain the protozoa in suspension at 39°C. Samples (1 ml) were removed periodically into 0.25 ml of 25% trichloracetic acid, and then the extract was centrifuged at 11,600 × *g* for 5 min. The supernatant fluid was counted by liquid scintillation spectrometry.

To determine the influence of removing tannins, polyvinylpyrrolidone was added to rumen fluid at a concentration of 2% (wt/vol) 1 h before labelled *S. ruminantium* was added (9). Saponins were extracted from the extract by using butanol in a procedure based on the methods of Headon et al. (16) and Wall et al. (30). Five milliliters of extract was shaken with 5 ml of *n*-butanol for 30 min at room temperature, the butanol was removed, and the extraction was repeated with 5 ml of fresh *n*-butanol. The butanol layers were pooled and dried in a rotary evaporator. The extracted solid was resuspended in 5 ml of water and added to the rumen fluid at a concentration of 1% (vol/vol). The residual aqueous layer was also tested at the same concentration for its antimicrobial effects. Purified saponins were obtained from Sigma and added to the rumen fluid 1 h before labelled bacteria.

Influence of *Y. shidigera* extract on growth of pure cultures of ruminal bacteria. *Butyrivibrio fibrisolvens* SH13, *S. ruminantium* Z108, and *Streptococcus bovis* ES1 were isolated from sheep rumina at the Rowett Research Institute. *Prevotella* (*Bacteroides*) *ruminicola* B_14 is a proteolytic strain isolated by Bryant et al. (2). The bacteria were grown in Hungate tubes containing either rumen fluid-containing medium 2 of Hobson (17) or the defined medium of Hungate and Stack (18). Growth was determined turbidimetrically at 650 nm in an LKB Nova spectrophotometer. *Y. shidigera* extract (1%, vol/vol), polyvinylpyrrolidone (2%, wt/vol), and resuspended butanol extract (1%, vol/vol) were added to the medium before autoclaving was performed.

Analyses. Dry weight was determined by drying 5 ml of extract at 80°C for 48 h. Protein was precipitated with 5% trichloroacetic acid and measured with the Folin reagent (21). Total carbohydrate was measured by using the phenol-sulfuric acid method (22). Nitrogen was determined by a Kjeldahl procedure (5). Saponins were estimated by hemolysis of sheep blood, with digitonin as a standard (1). Tannins were assayed by a radial diffusion method, using tannic acid in methanol solution as a standard (14). Total phenolics were measured by using the Folin reagent (27). Copper was determined by atomic absorption spectrometry, and propionate was determined by gas-liquid chromatography (26).

RESULTS AND DISCUSSION

Properties of *Y. shidigera* extract. The *Y. shidigera* extract supplied was a dark brown liquid with a specific gravity of 1.093 g/ml. Its dry weight was 284 mg/ml, its nitrogen content was 1.7 mg/ml, its protein content was 3.8 mg/ml, and it had a total carbohydrate content of 236 mg/ml. When the extract was dialyzed for 24 h against four changes of deionized water, these values were decreased to 49 mg (dry weight) per ml, 0.4 mg of N per ml, and 29 mg of carbohydrate per ml. Protein was not determined. The pH of a 1% solution of the original material was 4.1, probably because of the presence of 0.21 M propionic acid, but a 1% addition to rumen fluid decreased the pH of the fluid by only about 0.1 unit. Copper was present at a low concentration (0.82 ppm). Total phenolics were present at 16 mg/ml, and tannins were present at 12 mg/ml. The precipitation observed in the radial diffusion plates used for tannin estimation was much less distinct than that observed with tannic acid.

Ammonia-binding properties of *Y. shidigera* extract. When *Y. shidigera* extract was added to the phenol-hypochlorite ammonia assay, a concentration of 0.2 ml of *Y. shidigera* extract per ml of ammonia solution prevented color formation almost completely at ammonia concentrations of up to 0.4 mM (Fig. 1). One-tenth of a milliliter of extract per ml of solution gave about half the color intensity of controls. When the extract was neutralized to pH 7.0 with NaOH, it retained its effect on the ammonia assay. Similar observations were made previously (16), when it was demonstrated by other methods of ammonia determination that the decreased color formation was the result of ammonia binding to the extract rather than interference of the extract with the chemical reaction of ammonia analysis.

Thus, it might be concluded that the binding capacity of *Y. shidigera* extract at 0.4 mM ammonia was about 2 μmol of ammonia per ml of *Y. shidigera* extract. However, the inhibition of color formation was not consistent with classical saturation kinetics. As more ammonia was added, saturation did not occur, but rather a fairly constant proportion of ammonia remained undetected. Thus, the amount of ammonia bound appeared likely to increase proportionally as the concentration

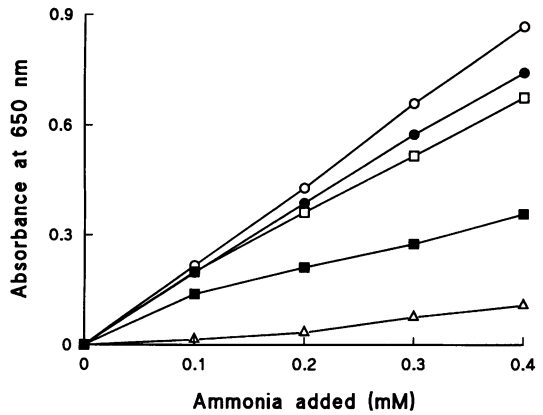

FIG. 1. Influence of *Y. shidigera* extract on the detection of ammonia by the phenol-hypochlorite-nitroprusside ammonia method. Extract was added per milliliter of ammonia-containing sample at 0.02 ml (●), 0.04 ml (□), 0.10 ml (■), and 0.20 ml (△). ○, no addition. Results are means of duplicate determinations.

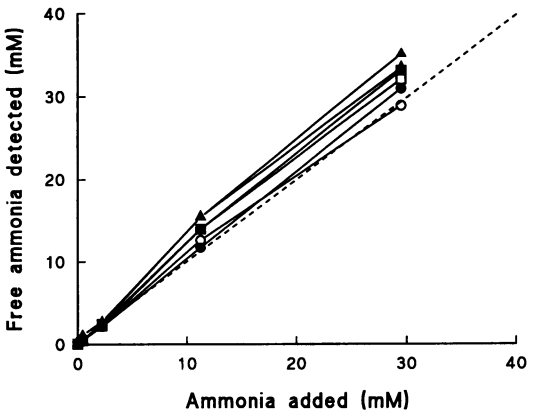

FIG. 2. Influence of *Y. shidigera* extract on free ammonia concentrations in 50 mM potassium phosphate buffer (pH 7.5). Increasing amounts of ammonia were added to buffer containing different concentrations of *Y. shidigera* extract, in which were suspended dialysis bags containing the same buffer but no extract. The different concentrations of extract added to buffer (in milliliters per liter) were 0.05 (●), 0.55 (□), 2.55 (■), 12.55 (△), and 62.55 (▲). ○, no addition. Ammonia analysis was done on the contents of dialysis bags. Results are means of duplicate incubations.

of ammonia increased, potentially giving the *Y. shidigera* extract a high capacity for ammonia adsorption if this ability were extrapolated to higher ammonia concentrations.

Furthermore, since any application of *Y. shidigera* extract in decreasing rumen ammonia would involve ammonia concentrations of 5 to 50 mM, the normal range found in the rumen, it was important to determine the ammonia-binding capacity of *Y. shidigera* extract at ammonia concentrations higher than could be used directly in the phenol-hypochlorite assay. This was done by incubating dialysis bags containing buffer with no *Y. shidigera* extract in larger volumes of buffer containing *Y. shidigera* extract to which were added increasing concentrations of ammonia. The bags could be removed and their contents could be diluted and analyzed without affecting the equilibrium of the *Y. shidigera* extract-ammonia interaction and without any interference of the nondialyzable, ammonia-binding component of the extract in the ammonia assay. One hour was allowed for the new equilibrium to be reached. No ammonia adsorption was seen at pH 7.5, even at *Y. shidigera* extract concentrations up to 62.55 ml/liter (Fig. 2). A similar pattern was observed at pH 2.0 (data not shown). Indeed, with both buffers, adding *Y. shidigera* extract appeared to increase the free ammonia concentration of the solution, indicating that the extract contained bound ammonia which was released into the dialysis bags. Thus, the ammonia-binding properties of *Y. shidigera* extract appeared to be detectable only at high ratios of extract/ammonia in dilute solutions of ammonia.

Dialyzing the extract caused a slight decrease in its ability to prevent color formation in the ammonia assay (Fig. 3), but autoclaving did not (Fig. 3), as was also found by Headon et al. (16). Thus, the ammonia-binding component is unlikely to be protein in nature. Headon et al. (16) have identified components (glycofractions) responsible for the ammonia binding.

Influence of *Y. shidigera* extract on ammonia concentrations and ammonia production in rumen fluid. *Y. shidigera* extract was added to rumen fluid from four sheep in vitro, and dialysis bags were removed at 3-h intervals to determine the influence of the extract on ammonia concentrations (Fig. 4). Urea and casein were added to rumen fluid in parallel incubations to determine the influence of *Y. shidigera* extract on rates of ammonia production (Fig. 4). Overall, *Y. shidigera* extract caused a 6% decrease in ammonia concentration ($P < 0.05$). *Y. shidigera* extract had no effect at 0 h ($P > 0.05$). Thereafter, the ammonia concentration was decreased ($P < 0.05$). This decrease was apparently not related to time of incubation ($P > 0.05$) nor was there a statistically significant interaction overall with either urea or casein ($P > 0.05$). However, there was a clear trend that the greatest effect of the *Y. shidigera* extract was in the rumen fluid with casein added at 6 h of incubation, whereas ammonia concentrations in the rumen fluid incubated with urea were unaffected.

It can therefore be concluded from these in vitro incubations that the acute effect of *Y. shidigera* extract on ammonia concentration in rumen fluid is small and most likely due to decreased proteolysis by the mixed population. However, the finding that any decrease occurred at all was of interest, in view of the earlier conclusions derived from tests with aqueous solutions that the ammonia-binding capacity of the extract

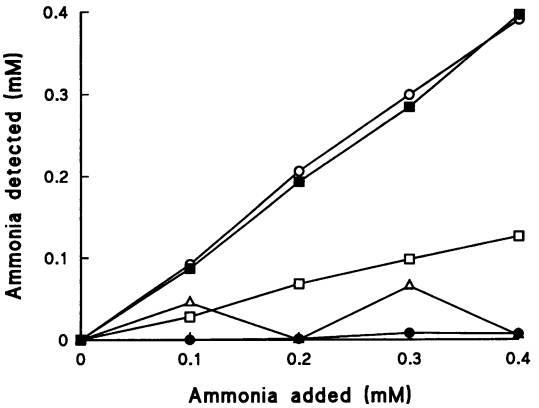

FIG. 3. Influence of autoclaved (△) and dialyzed (□) extract and extract precipitated with 5% trichloroacetic acid (■) on detection of ammonia by the phenol-hypochlorite-nitroprusside method. Control incubations were done in the absence of added extract (○) and with untreated extract (●). The volume of *Y. shidigera* extract or treated extract added was 0.2 ml per ml of ammonia-containing sample, as in Fig. 1. Results are means of duplicate determinations.

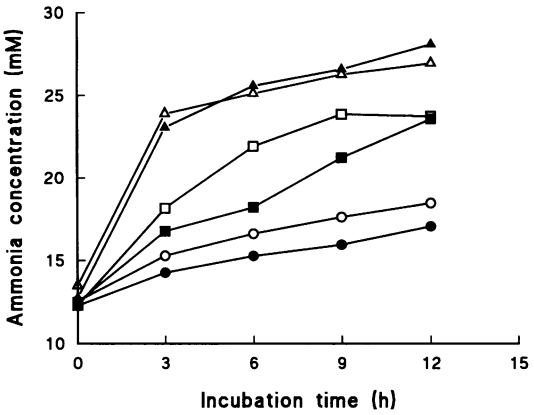

FIG. 4. Influence of *Y. shidigera* extract on free ammonia concentrations in rumen fluid incubated in vitro. Results are means of duplicate analyses from four samples of ruminal fluid from different sheep. Symbols: ○, no added N source; △, added urea (0.6 g/liter); □, added casein (4 g/liter). Open symbols indicate no *Y. shidigera* extract added; closed symbols indicate 1% (vol/vol) *Y. shidigera* extract added.

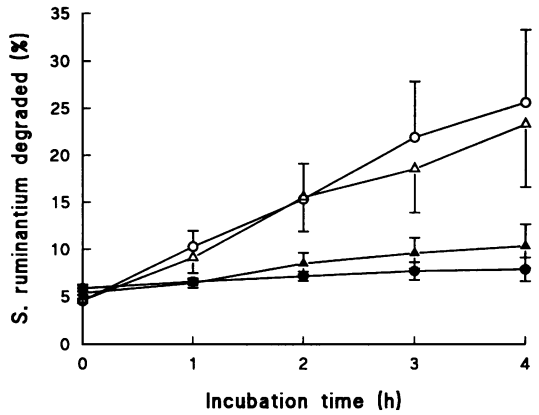

FIG. 6. Influence of butanol extraction on the inhibition by *Y. shidigera* extract of protozoal breakdown of [^{14}C]leucine-labelled *S. ruminantium* in strained rumen fluid in vitro. Symbols: ○, no addition; ●, 1% *Y. shidigera* extract; △, 1% butanol-extracted *Y. shidigera* extract; ▲, 1% reconstituted butanol extract of *Y. shidigera*. Results are the means and standard deviations for four sheep.

would be expected to be insignificant. An indirect influence on ammonia via antimicrobial effects was a possible explanation; therefore, the effects of *Y. shidigera* extract on ruminal microorganisms were determined.

Influence of *Y. shidigera* extract on ruminal ciliate protozoa. When *Y. shidigera* extract was added to rumen fluid in vitro and the effects on ciliate protozoa were observed microscopically, the cilia on the predominant species of both entodiniomorphs and holotrichs appeared to contract and motion was greatly reduced. To quantify the antiprotozoal effect, the rate of breakdown of [^{14}C]leucine-labelled *S. ruminantium* was used as an index of protozoal activity as described by Wallace and McPherson (31). Labelled leucine released from *S. ruminantium* is not reincorporated into microbial protein because an excess of cold leucine is added to the rumen fluid at the beginning of the incubation. *S. ruminantium* breakdown averaged 9%/h in the rumen fluid from four sheep (Fig. 5), in good agreement with rates observed before (31). The addition of 0.1% *Y. shidigera* extract decreased bacterial breakdown by 22%, and 1% extract caused bacterial breakdown to stop (Fig. 5). The addition of polyvinylpyrrolidone to remove tannins had no effect on bacterial breakdown (data not shown), but extraction of the extract with butanol removed the antiprotozoal effect (Fig. 6). The butanol extract, which contained more than 99% of the saponins but less than 8% of the total phenolic compounds, retained the antiprotozoal effect (Fig. 6).

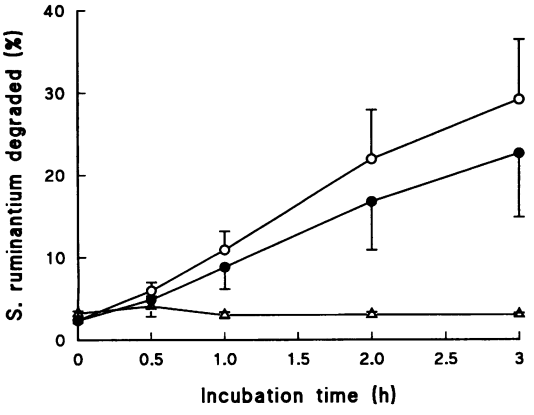

FIG. 5. Influence of *Y. shidigera* extract on protozoal activity in strained rumen fluid in vitro, as measured by the breakdown of [^{14}C]leucine-labelled *S. ruminantium*. Symbols: ○, no addition; ●, 0.1% *Y. shidigera* extract; △, 1% *Y. shidigera* extract. Results are the means and standard deviations for four sheep.

Saponins from *Quillaja* bark and *Saponaria* sp. were added to rumen fluid in an experiment of the same design as that illustrated by Fig. 6. After 4 h of incubation, *Quillaja* saponins caused decreases in breakdown of *S. ruminantium* of 27 and 49% at concentrations of 10 and 20 mg/ml, respectively. The equivalent decreases for *Saponaria* saponins were similar (25 and 43%, respectively). These values compare with a 91% decrease resulting from the addition of *Y. shidigera* extract with an estimated saponin content of 6.6 mg/ml. Without further fractionation, it is impossible to say whether there is a common antiprotozoal component which is present at different concentrations in the different saponins or whether entirely different components are involved in the extracts from the different plant species. Either way, *Y. shidigera* is potentially a source of powerful antiprotozoal saponins.

By far, the greatest cause of bacterial protein breakdown in the rumen is predation of bacteria by ciliate protozoa (31), and it is recognized that, where protein flow from the rumen limits animal production, removal of ciliate protozoa would improve the protein nutrition of the ruminant (19). The antiprotozoal effect of *Y. shidigera* saponins, if it were sustained in vivo, could be of nutritional usefulness. Evidence of a similar antiprotozoal effect was found in semicontinuous cultures of rumen microorganisms in which bacterial numbers increased and protozoal numbers declined in response to the addition of sarsaponin (28). The effects of *Y. shidigera* extracts on rumen fermentation appear inconsistent, as described in the introduction. It is possible that the variation is caused by the different contribution that protozoa make in different studies.

Influence of *Y. shidigera* extract on the growth of pure cultures of rumen bacteria. *Y. shidigera* extract added to the growth medium at a concentration of 1% stimulated the growth of *P. ruminicola* (Fig. 7). Growth of *P. ruminicola* was enhanced even more on Hungate and Stack defined medium (Fig. 7), suggesting that *Y. shidigera* extract had a nutritional

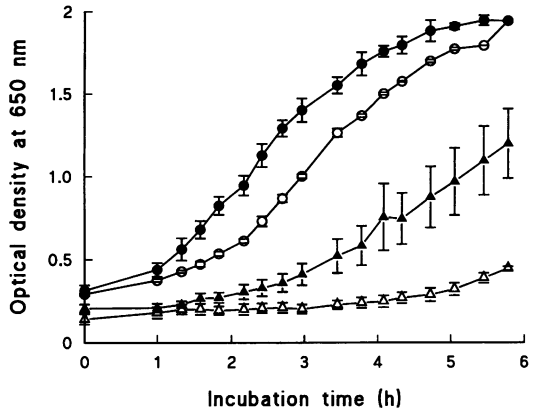

FIG. 7. Influence of *Y. shidigera* extract on the growth of the rumen bacterium *P. ruminicola*. Symbols: ○, Hobson's medium 2 (17); ●, Hobson's medium 2 plus 1% *Y. shidigera* extract; △, Hungate and Stack defined medium (18); ▲, Hungate and Stack defined medium plus 1% *Y. shidigera* extract. Results are means and standard deviations from three cultures.

effect. In contrast, growth of *S. bovis* was suppressed by *Y. shidigera* extract, which prolonged the lag phase following inoculation (Fig. 8). Growth of *B. fibrisolvens* was abolished, while that of *S. ruminantium* was unaffected (not shown). As with the protozoa, polyvinylpyrrolidone had no effect on the inhibitory properties of the preparation and the butanol extract contained the antibacterial component (data not shown). Thus, *Y. shidigera* extract appears in this brief survey to affect bacteria with a gram-positive ultrastructure more than gram-negative organisms. The antibacterial effects alone may therefore be beneficial by analogy with the effects of ionophores (3, 23, 25).

Conclusions. *Y. shidigera* extract has two components that might affect ammonia metabolism in the rumen. A glycocomponent, which is separate from the saponin fraction (16), binds ammonia but, on the basis of the binding capacity determined in the present experiments, this effect would not be expected to be significant at normal ruminal ammonia concentrations. On the other hand, the saponins of *Y. shidigera* may affect ammonia concentration indirectly via their toxicity to rumen ciliate protozoa and bacteria. Proteolysis was inhibited in in vitro incubations, resulting in slightly lower ammonia concentrations. A potentially significant effect is that saponins from *Y. shidigera* or other plants could be used to suppress ciliate protozoa, thereby improving the microbial yield in the rumen (7) and protein flow to the animal (19). A better characterization of the antiprotozoal factor and evaluation of the longer-term ability of saponins to suppress ciliate protozoa in vivo is now required.

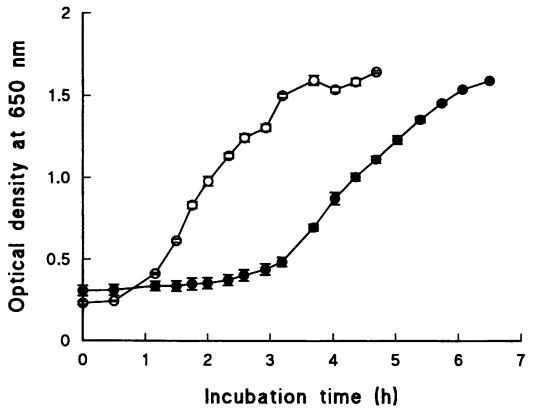

FIG. 8. Influence of *Y. shidigera* extract on the growth of the rumen bacterium *S. bovis*. Symbols: ○, Hobson's medium 2 (17); ●, Hobson's medium 2 plus 1% *Y. shidigera* extract. Results are means and standard deviations from three cultures.

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